Stem Cell Research 22 (2017) 61–69

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Proteolytic processed form of CXCL12 abolishes migration and induces apoptosis in neural stem cells in vitro

Taís Adelita [a], Roberta Sessa Stilhano [b], Sang Won Han [b], Giselle Zenker Justo [a,c], Marimelia Porcionatto [a,*]

[a] Department of Biochemistry, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, 04039, Brazil
[b] Department of Biophysics, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, 04044, Brazil
[c] Department of Cell Biology, Universidade Federal de São Paulo, Diadema, 09920, Brazil

ARTICLE INFO

Article history:
Received 11 November 2016
Received in revised form 5 April 2017
Accepted 24 May 2017
Available online 4 June 2017

Keywords:
CXCL12
CXCL12(5-67)
Adult neural stem cell
Migration
Apoptosis
In vitro study

ABSTRACT

The subventricular zone (SVZ) of the adult mammalian brain hosts full potential neural stem cells (NSCs). NSCs are able to respond to extracellular signals in the brain, amplifying the pool of progenitor cells and giving rise to neuroblasts that show ability to migrate towards an injury site. These signals can come from vascular system, cerebrospinal fluid, glial cells, or projections of neurons in adjoining regions. CXCL12, a chemokine secreted after brain injury, reaches the SVZ in a gradient manner and drives neuroblasts towards the lesion area. Among many other molecules, matrix metalloproteinase 2 and 9 (MMP-2/9) are also released during brain injury. MMP-2/9 can cleave CXCL12 generating a new molecule, CXCL12(5-67), and its effects on NSCs viability is not well described. Here we produced recombinant CXCL12 and CXCL12(5-67) and evaluated their effect in murine adult NSCs migration and survival in vitro. We showed CXCL12(5-67) does not promote NSCs migration, but does induce cell death. The NSC death induced by CXCL12(5-67) involves caspases 9 and 3/7 activation, implying the intrinsic apoptotic pathway in this phenomenon. Our evidences in vitro make CXCL12(5-67) and its receptor potential candidates for brain injuries and neurodegeneration studies.

1. Introduction

Resident neural stem cells (NSCs) persist in the adult mammalian central nervous system (CNS) and encourage the search for potential treatments for neurodegenerative and acute brain diseases. Full potential stem cells (type B or astrocytes-like cells) line in the subventricular zone (SVZ) along the wall of the lateral ventricles in the brain. These cells are capable of proliferate, increasing the pool of progenitor cells through the generation of transient amplifying cells (type C cells) (Alvarez-Buylla and Garcia-Verdugo, 2002; Doetsch et al., 1997). The SVZ neurogenic niche comprises many components such as the vascular system, extracellular matrix, microglia, astrocytes, neurons, and cerebrospinal fluid (CSF), representing a countless source of stimuli to NSCs (Falcao et al., 2012; Lim and Alvarez-Buylla, 2014; Walton et al., 2006). The type C cells can give rise to oligodendrocytes or generate immature ("blasts") neurons called neuroblasts or type A cells in vivo (Doetsch et al., 1999). Together with type B and type C cells, neuroblasts are generally referred as neural progenitor cells (NPCs) and migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB) (Lois and Alvarez-Buylla, 1994), where they differentiate into multiple types of interneurons (Lledo et al., 2008). During an injury, such as stroke (Arvidsson et al., 2002), traumatic brain injury (Ramaswamy et al., 2005), or neurodegenerative diseases (Saha et al., 2012), neuroblasts can migrate towards different areas of the CNS in response to signalling factors. Nevertheless, CNS regeneration process must overcome many obstacles besides NSCs expansion and migration, such as survival, differentiation into specific neural subtypes, and integration into a pre-existing neural network. Despite this capacity of neuroblasts to generate neurons in different CNS areas, the majority of them undergo apoptosis when arriving at a lesion site, resulting in absent or poor regeneration of adult mammalian brain (Arvidsson et al., 2002; Malone et al., 2012; Thored et al., 2006).

After a brain injury, soluble factors are released at the lesion site, reaching the SVZ through blood vessels, parenchymal diffusion or cell-cell communication. These factors provide cues that direct neuroblasts to the damaged areas. The chemokine CXCL12 (C-X-C motif ligand 12) – which also regulates homing and maintenance of stem cells in the niches – is among these factors (Kokovay et al., 2010). CXCL12, previously known as SDF-1 (stromal cell-derived factor 1), is a small secreted chemotactic cytokine composed of 67 amino acids. The N-terminus amino acid sequence of CXCL12 (KPVSLSYR, amino acids 1 to 8) (Fig. 1a) is critical for receptor activation and the sequence RFFESHI (amino acids 12 to 18) promotes the initial docking of the chemokine to its receptor CXCR4 (Crump et al., 1997). CXCL12 is abundant and selectively expressed in the developing and mature CNS (Tham et al., 2001), and is

* Corresponding author.
E-mail address: marimelia.porcionatto@unifesp.br (M. Porcionatto).

http://dx.doi.org/10.1016/j.scr.2017.05.013

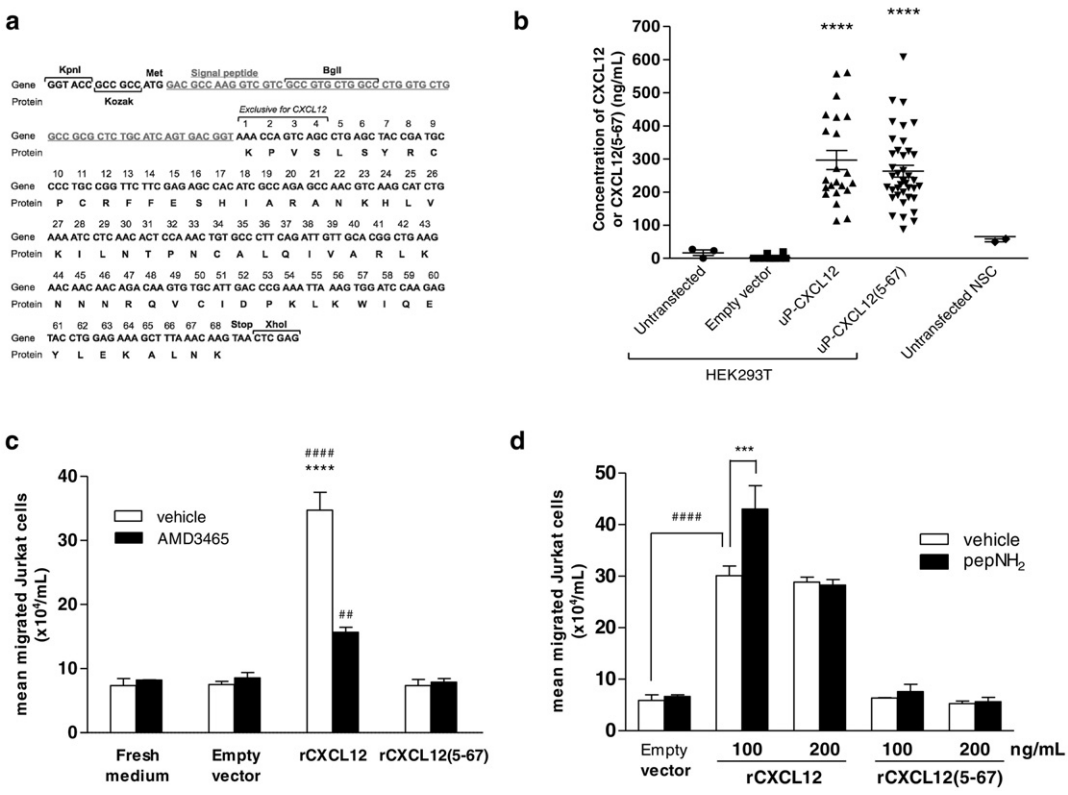

Fig. 1. Recombinant CXCL12 and CXCL12(5-67) production and their effect in Jurkat cells chemotaxis. (a) Nucleotides sequences inserted in the uP vector to produce rCXCL12 and rCXCL12(5-67). CXCL12 *Mus musculus* sequence was used as reference to synthesize the recombinant chemokines and KOZAC sequence was inserted before the signal peptide. *Kpn*I and *Xho*I restriction enzyme sites are flanking the coding sequence. *Bgl*I recognition site is identified and was used to check the insert presence in the vectors. CXCL12(5-67) lacks the first four amino acids from the full length chemokine (K, lysine; P, proline; V, valine; S, serine). (b) Level of rCXCL12 or rCXCL12(5-67) secreted by HEK293 and NSCs tested by ELISA. Untransfected: 1.9, SEM ± 23.2, $n = 3$; Empty vector: −49.3, SEM ± 12.9, $n = 10$; uP-CXCL12: 296.9, SEM ± 28.9, $^{****}P < 0.0001$ uP-CXCL12 vs. untransfected, $n = 22$; uP-CXCL12(5-67): SEM ± 17.8, $^{****}P < 0.0001$ uP-CXCL12 vs. untransfected, $n = 39$; Untransfected NSC: 54.3, SEM ± 4.3, $n = 2$. (c) Jurkat cells chemotaxis in the presence of 50 ng/mL of rCXCL12 or rCXCL12(5-67) and controls. The chemotactic activity was also determined in the presence of CXCR4 antagonist (AMD3465). Fresh medium: 7.3, SEM ± 1.1, and 8.2, SEM ± 0.04; Empty vector: 7.5, SEM ± 0.5, and 8.5, SEM ± 0.08; rCXCL12: 34.7, SEM ± 2.8, and 15.67, SEM ± 0.8, $^{****}P < 0.0001$ rCXCL12 vehicle vs. rCXCL12 AMD3465, $^{\#\#\#\#}P < 0.0001$ rCXCL12 vehicle vs. Fresh medium vehicle, and $^{\#\#}P = 0.0042$ rCXCL12 AMD3465 vs. Fresh medium AMD3465. Vehicle and AMD3465 values were presented in that order. $N = 3$. (d) Jurkat cells chemotaxis in the presence of 100 or 200 ng/mL of rCXCL12 or rCXCL12(5-67). The chemotactic activity was also determined in the presence of CXCL12 N-terminal peptide [KPVSLSYR-NH_2 (pep-NH2)]. Empty vector: 5.9, SEM ± 1.0, and 6.7, SEM ± 0.3; 100 ng/mL rCXCL12: 30.1, SEM ± 1.9, and 43.0, SEM ± 4.5, $^{***}P = 0.0003$ rCXCL12 vehicle vs. rCXCL12 pep-NH2, $^{\#\#\#\#}P < 0.0001$ rCXCL12 vehicle vs. Empty vector vehicle, and $^{\#\#\#\#}P < 0.0001$ rCXCL12 pep-NH2 vs. Empty vector pep-NH2; 200 ng/mL rCXCL12: 28.8, SEM ± 1.0, and 28.3, SEM ± 1.1, $^{\#\#\#\#}P < 0.0001$ rCXCL12 vehicle vs. Empty vector vehicle, and $^{\#\#\#\#}P < 0.0001$ rCXCL12 pep-NH2 vs. Empty vector pep-NH2. 100 ng/mL rCXCL12(5-67): 6.3, SEM ± 0.1, and 7.6, SEM ± 1.4; 200 ng/mL rCXCL12(5-67): 5.3, SEM ± 0.5, and 5.7, SEM ± 0.8. Vehicle and pep-NH2 values were presented in that order. $N = 3$. *Two-way or #one-way ANOVA.

secreted by endothelial cells, astrocytes, microglia and neurons (Banisadr et al., 2003). CXCR4 (C-X-C motif receptor 4) is the signalling G protein-coupled receptor of CXCL12, also widely expressed in the CNS (Banisadr et al., 2002).

The CXCL12/CXCR4 axis is involved in mobilization, proliferation, migration and differentiation of progenitor cells mainly during development but also in adulthood (Bajetto et al., 1999; Imitola et al., 2004; Itoh et al., 2009; McGrath et al., 1999; Tiveron et al., 2006). Constitutive expression of CXCL12 in the adult CNS is kept at a low level, and is upregulated under injury states, when then CXCL12 enhances the recruitment of neuroblasts from the SVZ neurogenic niche to lesion sites and provides signalling for a potential endogenous stem cell-based repair (for a review see Li et al., 2012).

Partial cleavage of CXCL12 at the N-terminus by distinct peptidases results in loss of chemotactic activity and impairment in CXCR4 receptor affinity (Cho et al., 2010; Delgado et al., 2001; Levesque et al., 2003). The proteolytic cleavage of CXCL12 by matrix metalloproteinase 2 and 9 (MMP-2 and 9) removes selectively the first four N-terminus amino acids from the full length molecule, generating the truncated form CXCL12(5-67) (McQuibban et al., 2001). Previous work (Denoyer et al., 2012; Van Raemdonck et al., 2014; Vergote et al., 2006; Zhu et al., 2009) showed that CXCR3, natural receptor of CXCL9, 10 and 11, acts as signalling receptor for CXCL12(5-67). These authors also demonstrated in those independent studies that CXCL12(5-67) affects the viability of differentiated cell types in the CNS, but its effect on neural stem cells remains poorly studied.

The aim of the present work was to investigate the activity of the truncated form of CXCL12, CXCL12(5-67), in NSCs viability. Herein we produced CXCL12 and CXCL12(5-67) recombinant and assessed their activity on adult murine NSCs migration and viability in vitro. Our data show, for the first time, that CXCL12(5-67) induces apoptosis in adult NSCs in vitro. The demonstration of an emerging role for CXCL12(5-67) in the low regenerative capacity of the CNS provides a basis for considering this cleaved form of CXCL12 as a novel target for treatment during traumatic brain and neurodegenerative diseases.

2. Results

2.1. CXCL12(5-67) is not chemotactic to CXCR4$^+$ cells

We constructed uP vectors containing either the native or the cleaved form of CXCL12 sequence and transfected HEK293T cells in order to produce CXCL12 and CXCL12(5-67) (Fig. 1a). Both forms of recombinant CXCL12 [rCXCL12 and rCXCL12(5-67)] were secreted in HEK293T conditioned medium. The concentration obtained was on average 296.9 ng/mL for rCXCL12 and 263.1 ng/mL for rCXCL12(5-67) (Fig. 1b). HEK293T cells transfected with empty uP vector and untransfected did not secret detectable amounts of CXCL12.